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(54) Title: NUCLEOSIDE ANALOGUES

(57) Abstract

Nucleoside analogues containing ribofuranosyl β-oriented fused heterocyclic and 3'-spiro substituents are provided for the treatment or prophylaxis of retroviral infections. Novel compositions containing such analogues are administered to subjects in order to repress retroviral reverse transcription.

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NUCLEOSIDE ANALOGUES

This invention relates to methods for the therapy and prophylaxis of infections, in particular to infections by retroviruses such as HIV. More specifically, this invention relates to the use and preparation of nucleoside analogues to treat such infections.

Several deoxyribonucleoside analogues are known to be potent inhibitors of retroviral reverse transcriptase (RT), in particular the RT of human immunodeficiency virus (HIV), the virus known to be the causative agent of acquired immunodeficiency syndrome (AIDS)[1]. These analogues [e.g., 3'-azido-3'-deoxythymidine (AZT) and 2',3'dideoxycytidine (ddC)] are converted in vivo by cellular kinases to the active 5' triphosphates. The triphosphates are the active agents in inhibiting RT. These triphosphates are recognized by RT as substrates and are thought to be incorporated into DNA. Since they lack a 3'-hydroxyl for elongation of the DNA chain, and since RT has no 3'-5' proofreading ability, DNA synthesis is irreversibly halted. Analogues such as AZT or ddC have properties which make them valuable as AIDS therapeutic agents; that is, the corresponding triphosphates are much better substrates for RT than they are for the cellular DNA polymerase (Pol α). This fact means that these analogues can selectively inhibit RT without halting host DNA synthesis[1].

The rational design of nucleoside analogue inhibitors of RT is limited in several regards; the major limitations are due to the fact that the exact

molecular mechanisms of the substrate recognition and catalysis by cellular kinases, RT and Pol α are not known. The reports which have led to the use of AZT and ddC as AIDS therapies followed the synthesis of these compounds by more than a decade[1]. These compounds were taken "off the shelf" and applied as AIDS therapies. Thus the nucleoside analogues which are proposed or in use for therapy or prophylaxis of AIDS infection were not designed for that purpose. They exert side effects, e.g. bone marrow toxicity, and are not sufficiently effective to be considered a therapeutic cure. In addition, AZT in particular has been reported to be extremely costly because of its complex synthetic route. Finally, known nucleoside analogues for treatment of retroviral infections act by inducing DNA chain termination, but other mechanisms of action for retroviral RT inhibition or inactivation have not been explored.

Accordingly, it is an object of this invention to reduce or eliminate the toxicity and side-effects of nucleoside analogues at therapeutic dosages.

It is another object to improve the efficacy of nucleoside analogues in the treatment or prophylaxis of retroviral infections.

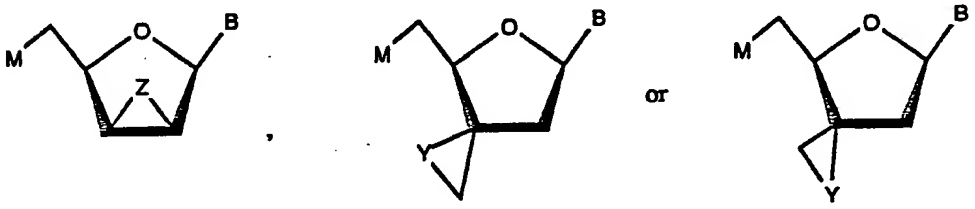
It is an additional object to provide nucleoside analogues which are less expensive to synthesize than those which are currently in use for the treatment of HIV.

It is a still further object to provide reactive nucleoside analogues which are capable of functioning as suicide substrates for RT.

These and other objects will be apparent to the ordinary artisan from the specification as a whole.

Summary of the Invention

Objects of this invention are accomplished by a method comprising administering to a subject a therapeutically effective dose of a compound selected from the following groups and their pharmaceutically acceptable salts:



Z is O, S or NR;

Y is O or NR;

R is H or acyl;

B is a purine or pyrimidine base other than uracil, or an analogue of such base which is capable of ambiguous base pairing; and

M is hydroxyl or an ester; provided, however, that when Z is O then B is not 5-bromouridinyl, thyminyl, O^4-methyluracil, N^4-hydroxycytosinyl, or N^4-methylcytosinyl.

These compounds are useful for anti-infective therapy, including the treatment of retroviral infections. They are formulated into pharmaceutically effective dosage forms for administration to patients, particularly patients infected with HIV.

Detailed Description of the Invention

In general, and with the exceptions noted above, "B" is any purine or pyrimidine base, or analogue thereof, other than uracil, which is capable of base pairing with polynucleotide cytosine, adenine, thymine, uridine or guanine bases. B is linked to the sugar 1' site through the 9- position of purines or 1- position of pyrimidines. Preferably, base B is cytosinyl or thyminyl, although other bases are suitable. For example, B is selected from among thyminyl, cytosinyl, N^4- substituted cytosinyl, O^4-substituted uridinyl and 5- substituted thyminyl. O^4-uridinyl substituents include methyl and -OH, and 5-uridinyl substituents include replacement of the 5 hydrogen with halogen, e.g. bromine. Analogues of the purine bases adenine or guanine include 2,6-diaminopurine, 6-methylthiopurine, 6-methoxypurine, xanthosine, hypoxanthine, purine and 2-amino purine. The nature of the base is not critical so long as it is able to confer on the nucleoside analogue of which it is a part the ability to base pair with a polynucleotide, preferably RNA, under the aegis of RT. Base B of the nucleoside analogues herein will be selected on the basis of being incorporated into DNA by RT or being reactive with RT to a greater degree than with mammalian Pol-α, as is readily determined by in vivo or in vitro screening.

The pentofuranosyl moieties fall within two classes: the beta (or upper) oriented epoxides, episulfides and aziridines, and the 3' spiro epoxides and aziridines.

Preferably, the beta epoxides are employed. The aziridines are expected to be less stable in vivo than

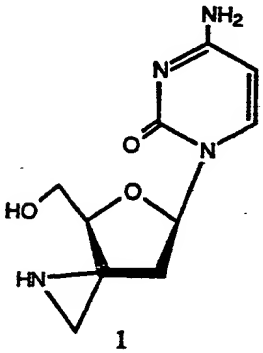
the epoxid s and episulfide but off r promise as "suicide" substrates for RT. In this scenario, and without limiting the invention to any particular theory of action, nucleophilic attack by an amino acid side chain of RT will result in acylation of the enzyme, in turn inhibiting or inactivating the enzyme. The acyl groups "R" preferably are acetyl or formyl since these offer the least steric bulk. However, other acyl substituents also are believed to be usable.

M is preferably hydroxyl. However, esters are also suitable, particularly those which are hydrolyzed in vivo to yield 5' hydroxyl. Such esters are useful in sustained release formulations wherein endogenous esterolytic enzymes gradually release the 5' hydroxyl species. Esters include carboxylic acid esters in which the non-carbonyl moiety of the ester grouping is selected from straight or branched chain alkyl (about 1-18 carbon atoms, preferably 1-4), alkoxyalkyl (e.g. methoxymethyl), aralkyl (e.g. benzyl), arloxyalkyl (e.g. phenoxymethyl), aryl (e.g. phenyl optionally substituted by halogen, C_{1-4} alkyl or C_{1-4} alkoxy); and sulphonate esters such as alkyl- or aralkylsulphonyl (e.g. methanesulphonyl). Mono-, di-, or triphosphate esters are not preferred because they are not believed to cross cell membranes as readily as compounds having more hydrophobic 5' substituents, although it will be appreciated that cellular protein kinases will triphosphorylate the 5' hydroxyl, thereby creating the chain terminating species.

Any reference to any of the compounds herein also includes the pharmaceutically acceptable salts of such compounds. Examples of pharmaceutically acceptable salts include those of alkaline earths (e.g. sodium or

magnesium), ammonium or NX_4^+ (wherein X is C_{1-4} alkyl). Other pharmaceutically acceptable salts include organic carboxylic acids such as acetic, lactic, tartaric, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-tolunesulfonic acids and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound having a hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+, NH_4^+, and NX_4^+ (wherein X is a C_{1-4} alkyl group).

Examples of compounds falling within the scope herein have the structures



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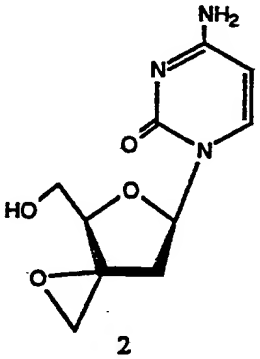

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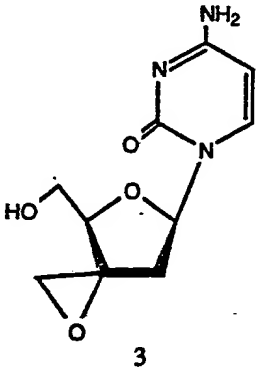

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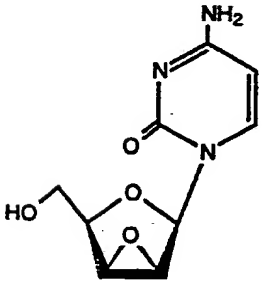

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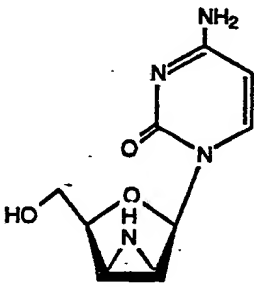

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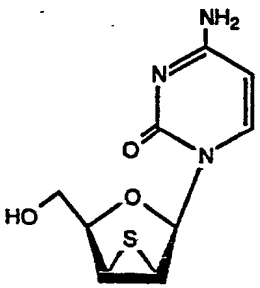

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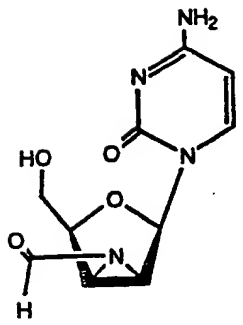

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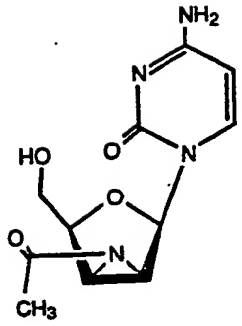

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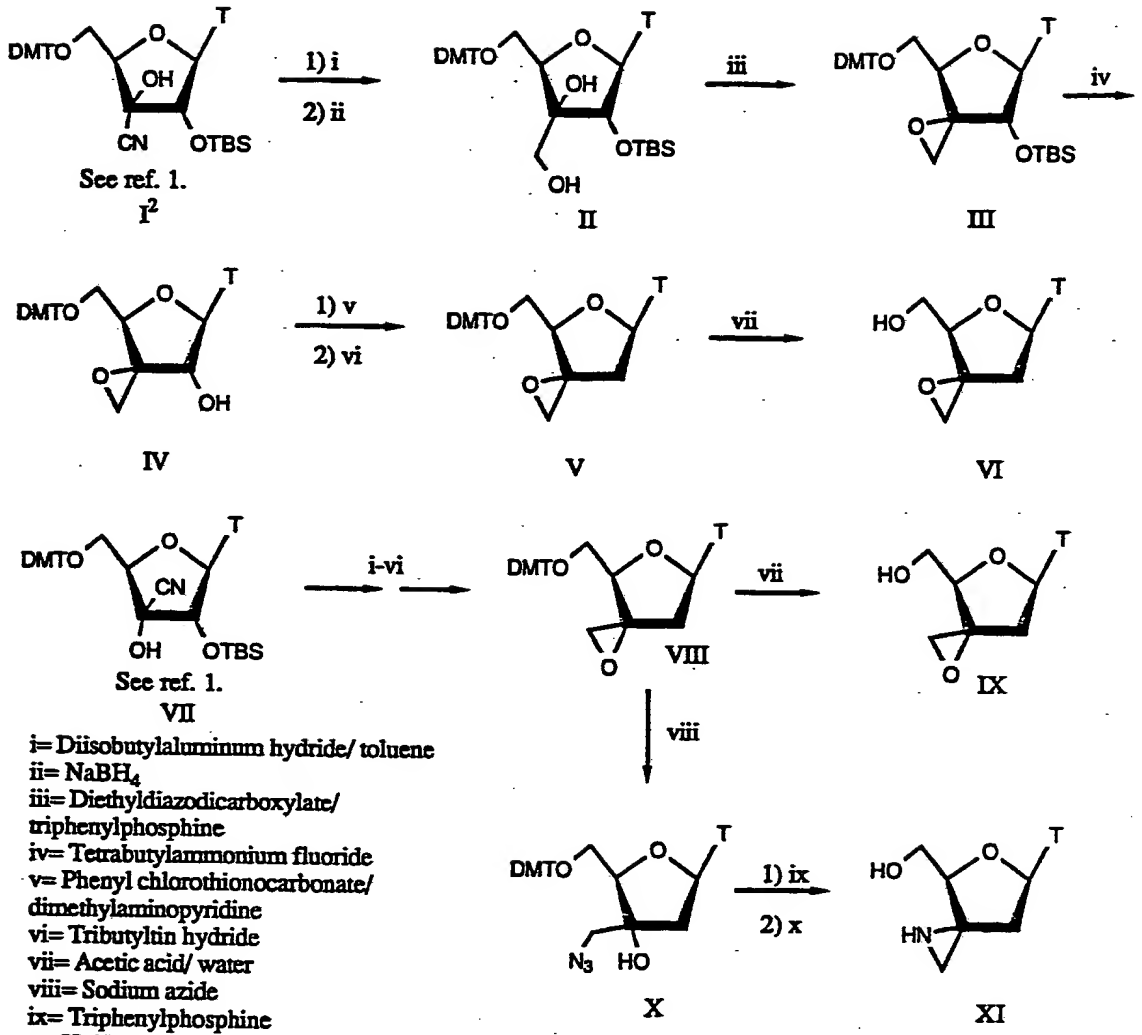
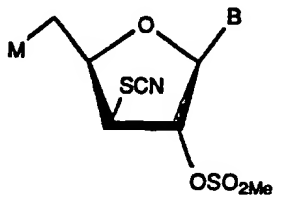
The compounds of this invention are prepared by methods known in the art or modifications thereof as will be apparent to the ordinary artisan. Compound 4 is prepared by the method of Hollenberg et al.[5] Compound 5 may be prepared by methods available to the art. However, the action of lithium azide on 5'-dimethoxytrityl-3'-mesyluridine followed by removal of the trityl group and treatment of the resulting trans azido alcohol with triphenylphosphine failed to give 5. Compound 5 is converted into 7 or 8 by treatment with the appropriate acid anhydride. Compounds 1 to 3 (designated XI, VI and IX respectively) are made using the following procedure.



i= Diisobutylaluminum hydride/ toluene
ii= $NaBH_4$
iii= Diethyldiazodicarboxylate/ triphenylphosphine
iv= Tetrabutylammonium fluoride
v= Phenyl chlorothionocarbonate/ dimethylaminopyridine
vi= Tributyltin hydride
vii= Acetic acid/ water
viii= Sodium azide
ix= Triphenylphosphine
x= H_2/ Pd

References;
1) A. Calvo-Mateo, M. Camarasa, A. Diaz-Ortiz and F. G. De las Heras, Tetrahedron Lett., 29, 941-944, 1988.
2) T. R. Webb, H. Mitsuya, and S. Broder, J. Med. Chem., in press 1988.

The synthesis of 6 may be accomplished by treating 5'-dimethoxytrityl-2',3'-dimesyl-uridine with Na_2S or sodium thioacetate followed by mild base treatment, followed by conversion of the uracil base to a cytosine base by known methods. Alternatively the episulfide can be prepared by known methods, see "Advanced Organic Chemistry", Jerry March, Wiley Interscience, New York (1985) or by modification of the method set forth in Japanese patent application 103,509 (filed 8/28/75) wherein the substituents at the 2' and 3' position of the starting material have the opposite orientation from that which is shown in the application, i.e.



The synthesis of purine-β-2',3'epoxynucleosides can be accomplished according to the procedure of Robins, M.J. et al.[4]

Esters at M include monophosphates; diphosphate; triphosphate; acetate; 3-methyl-butyrate; octanoate; palmitate; 3-chloro benzoate; benzoate; 4-methyl benzoate; hydrogen succinate; pivalate; and mesylate.

The compounds according to the invention are administered for therapy of infectious agents by any suitable route including oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous and intradermal). It will be appreciated that the preferred route and dosage will vary with the

condition and age of the recipient, the infection involved, side effects such as anemia, the clinical condition of the patient, the identity of the infectious agent and other parameters which the skilled clinician typically encounters.

The compounds according to the invention are active against viruses, in particular retroviruses such as lymphotropic viruses (HTLV), especially HTLV-I, HTLV-II and HIV. The invention accordingly provides the compounds according to the invention for use in the treatment or prophylaxis of the above infections.

In general a suitable dose will be in the range of 1.0 to 50 mg per kilogram body weight of the recipient per day, preferably in the range of 3 to 30 mg per kilogram body weight per day and most preferably in the range of 5 to 15 mg per kilogram body weight per day. The desired dose is preferably presented as two, three, four, five, six or more sub-doses administered at appropriate intervals throughout the day, or is administered by continuous i.v. pump. Sub-doses may be administered in unit dosage forms, for example, containing 2 to 25 mg, preferably 3 to 20 mg, and most preferably 4 to 10 mg of active ingredient per unit dosage form. The active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 1 to about 100 μM, preferably about 3 to 50 μM, most preferably about 5 to about 15 μM. The therapeutic amounts of compounds having Z = S or NR, or Y=O or NR are expected to be less than required for the remaining compounds, and lower than the ranges set forth in this paragraph.

While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. The formulations of the present invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers thereof and optionally other therapeutic agents. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. providone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach. This is particularly advantageous where, B is a purine and/or Z is NH or S since such compounds are susceptible to acid hydrolysis.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pasts, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, minibags, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

The compounds according to the invention may also be presented for use in the form of veterinary formulations, which may be prepared, for example, by methods that are conventional in the art. Examples of such veterinary formulations include those adapted for:

(a) oral administration, external application, for example drenches (e.g. aqueous or non-aqueous

solutions or suspensions); tablets or boluses; powders, granules or pellets for admixture with feed stuffs; pastes for application to the tongue;

(b) parenteral administration for example by subcutaneous, intramuscular or intravenous injection, e.g. as a sterile solution or suspension; (when appropriate) by intramammary injection where a suspension or solution is introduced into the udder via the teat;

(c) topical application, e.g. as a cream, ointment or spray applied to the skin; or

(d) intravaginally, e.g. as a pessary, cream or foam.

The administered ingredients may also be used in therapy in conjunction with other medicaments such as antibiotics, 9-[[2-hydroxy-1-(hydroxy-methyl)ethoxy]methyl]guanine, 2-amino-9-(2-hydroxyethoxymethyl)purine, interferon, e.g., γ or α interferon, tumor necrosis factor, interleukin II, AZT, ddC and phosphonoformate, as is appropriate. In one treatment embodiment, retroviral replication cycles are induced by immunostimulants such as bacterial peptides or lymphokines, during which period the compounds of the invention are administered to the subject, optionally together with tumor necrosis factor. Thereafter, an intermediate stage without immunostimulation and, optionally, coupled with compound administration is permitted to pass, after which the compound treatment cycle is repeated.

EXAMPLE 1

Compound 4, AZT and ddC were diluted into PBS and used to produce solutions of cell culture medium in which the concentrations of 4 were 1, 10 and 100 μM 1, 5 and 50 μM (AZT) and 0.1 and 10 μM (ddC). The effect of these compounds on the growth of HIV infected ATH8 cells (2 x 10^5 cells per tube; 2000 virus particles/cell) was determined by the method of Broder et al.[1] A compound control contained the candidate compound but the cells were not virally infected. A treatment control contained no compound nor were the cells infected with virus. Viable cells were counted in each tube after six days incubation. Percent protection was equal to 100 x (the number of cells surviving the viral infection divided by the number of cells in the compound control). The percent toxicity was equal to 100 x (the number of surviving cells in the compound control divided by the number of cells in the treatment control).

The results are shown in the table below.

TABLE

Candidate	Concentration (μM)	Protection (%)	Toxicity (%)
AZT	1, 5, 50	78, 98, 47	3, 11, 50
ddC	0.1, 1, 10	12, 119, 96	0, 0, 4
6	1, 10, 100	20, 100, 50	0, 30, 50

This demonstrates that compound 4 confers substantial protection against HIV infection and is substantially nontoxic at concentrations below about 5 μM. The adenyl derivative of compound 4 was comparatively weakly active in conferring protection.

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2. A. Calvo-Mateo, M. Camarasa, A. Diaz-Ortiz and F.G. De las Heras, Tetrahedron Lett. 29:941-944 (1988).

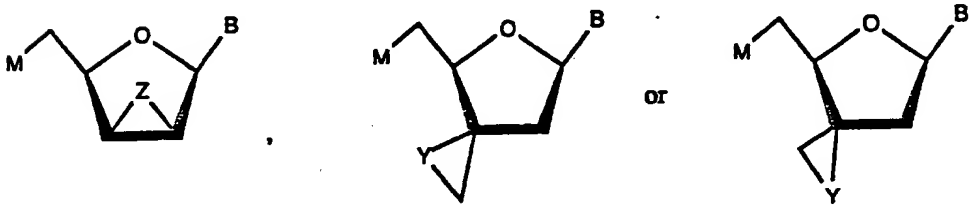
3. Kim, C. Marquez, V.E., Broder, S., Mitsuya, H. and Driscoll, J.S. "J. Med. Chem." 30:862-866 (1987).

4. Robins, M.J.; Hansske, F.; Low, N.H.; Park, J.I.; "Tet. Lett." 25:367 (1984).

5. Hollenberg , D.; Watanabe, K.; Fox, J. "J. Med. Chem. 41:2042 (1976).

Claims:

1. A method for anti-infective therapy comprising administering to a subject a therapeutically effective dose of a compound selected from the following groups and their pharmaceutically acceptable salts:



Z is O, S or NR;

Y is O or NR;

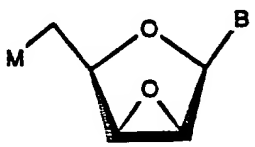
R is H or acyl;

B is a purine or pyrimidine base other than uracil, or an analogue of such base which is capable of ambiguous base pairing; and

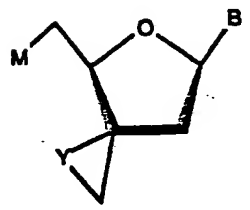
M is hydroxyl or an ester; provided, however, that when Z is O then B is not 5-bromouridinyl, thyminyl, O^4-methyluracil, N^4-hydroxycytosinyl, or N^4-methylcytosinyl.

2. The method of claim 1 wherein the subject is infected with HIV.

3. The method of claim 1 wherein the compound is



4. The method of claim 1 wherein the compound is



5. The method of claim 3 wherein B is cytosinyl or adenyl.

6. The method of claim 4 wherein B is cytosinyl or adenyl and Y is O.

7. The method of claim 1 further comprising administering to the subject an interferon and a tumor necrosis factor.

8. The method of claim 1 wherein a plurality of said compounds are administered to the subject.

9. The method of claim 7 wherein the compound is administered at substantially the same time as the interferon, and tumor necrosis factor is administered thereafter.

10. The method of claim 1 comprising stimulating the immune system of the patient while administering the compound, followed by withdrawing the immune stimulus.

11. The method of claim 10 which is repeated through a plurality of cycles.

12. The method of claim 1 wherein the dose is sufficient to produce a plasma concentration of the compound in the subject ranging about from 1 to 50 μM.

13. The method of claim 12 wherein the dose is sufficient to produce a plasma concentration of about from 1 to 15 μM.

14. The method of claim 4 wherein Y is NH, M is hydroxyl and B is cytosinyl.

15. The method of claim 3 wherein M is hydroxyl and B is cytosinyl.

16. The method of claim 1 wherein Z is S, M is hydroxyl and B is cytosinyl, thyminyl, adenyl or guanyl.

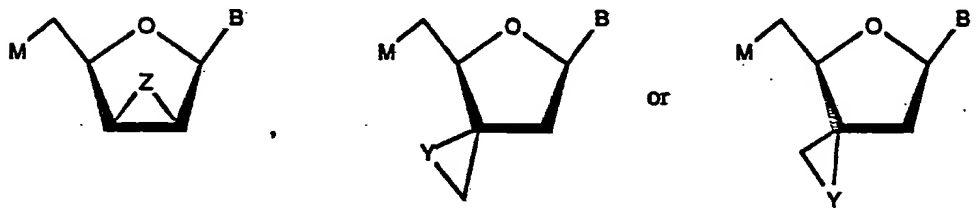
17. The method of claim 1 wherein Z is NR, M is hydroxyl and B is a purine or pyrimidine base or an analogue thereof which is capable of ambiguous base pairing, other than cytosine, thymine, adenine, or guanine.

18. The method of claim 17 wherein R is hydrogen.

19. The method of claim 17 wherein B is N^4-substituted cytosine, O^4-substituted uracil, 5-substituted uracil, 2,6-diaminopurine, 6-methylpurine, 6-methoxypurine, xanthosine, hypoxanthine, 2-amino purine and purine.

20. The method of claim 4 wherein Y is O.

21. A compound selected from the following groups and their pharmaceutically acceptable salts:



Z is O, S or NR;

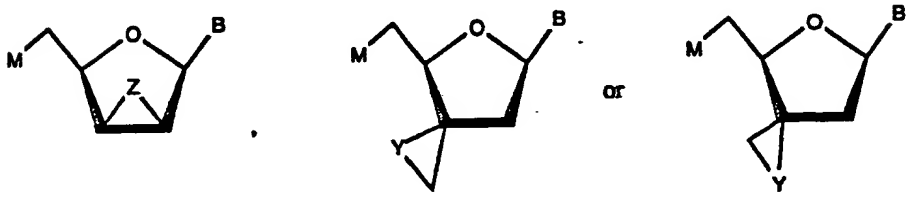
Y is O or NR;

R is H or acyl;

B is a purine or pyrimidine base other than uracil, or an analogue of such base which is capable of ambiguous base pairing; and

M is hydroxyl or an ester; provided, however, that when Z is O then B is not 5-bromouridinyl, thyminyl, O^4-methyluracil, N^4-hydroxycytosinyl, or N^4-methylcytosinyl; further excluding, however, those compounds wherein Z is O, B is cytosine, 5-methyl uracil, or adenosine, and M is hydroxyl.

22. A pharmaceutical preparation comprising a physiologically innocuous carrier and a compound selected from the following groups and their pharmaceutically acceptable salts:



Z is O, S or NR;

Y is O or NR;

R is H or acyl;

B is a purine or pyrimidine base other than uracil, or an analogue of such base which is capable of ambiguous base pairing; and

M is hydroxyl or an ester; provided, however, that when Z is O then B is not 5-bromouridinyl, thyminyl, O^4-methyluracil, N^4-hydroxycytosinyl, or N^4-methylcytosinyl.

23. The preparation of claim 22 which is sterile.

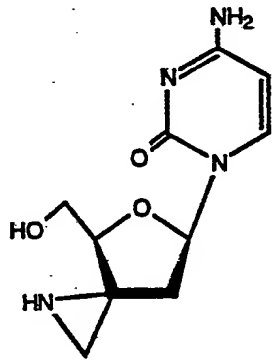
24. The preparation of claim 22 which is packaged into a unitary dosage form.

25. The preparation of claim 24 wherein the unitary dosage form is a tablet or capsule.

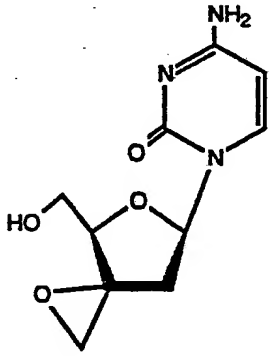
26. The preparation of claim 22 wherein the carrier is substantially isotonic.

27. The preparation of claim 22 wherein the carrier is a sustained release polymer.

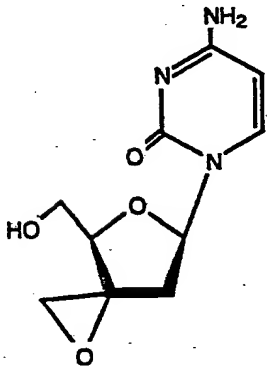
28. The compound



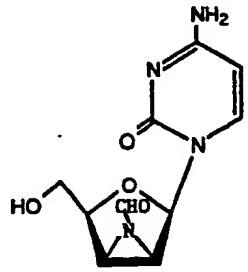
29. The compound



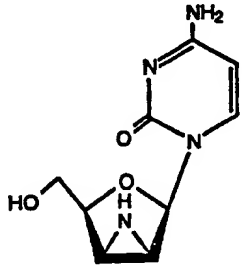
30. The compound



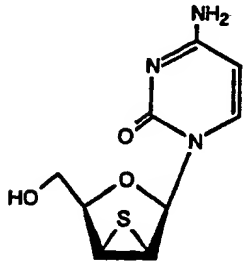
31. The compound



32. The compound



33. The compound



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/01812

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 07 H 19/06; A 61 K 31/70

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 07 H 19/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Chemical Abstracts, vol. 87, 1977 (Columbus, Ohio, US) Ueda, Tohru et al.: "Nucleosides and nucleotides. XV. Synthesis of 2',3'-episulfides derived from uridine" see page 580, abstract no. 68571p, & J. Carbohydr. Nucleosides. Nucleotides 1976, 3(5-6), 365-8 --	21,33
X	Chemical Abstracts, vol. 87, 1977, (Columbus, Ohio, US) see page 657, abstract no. 102602m & JP, A, 7727780 (UEDA, TORU et al.) 2 March 1977 --	21,33
X	Journal of Medicinal Chemistry, vol. 20, no. 1, 1977 (Columbus, Ohio, US) D.H. Hollenberg et al.: "Nucleosides. 102. Synthesis of some 3'-deoxy-3'-substituted arabinofuransosylpyrimidine nucleosides", pages 113-116 see compound 5a,b	21

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
8th September 1988	22 SEP 1988
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

III. DOCUMENTS C NSIDERED TO BE RELEVANT (CONTINUED FR M THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Journal of Organic Chemistry, vol. 39,no. 11, 1974 (Columbus, Ohio, US) M.J. Robins et al.: "Nucleic acid related compounds. 11. Adenosine 2',3'-ribo-epoxide. Synthesis, intramolecular degradation, and transformation into 3'-substituted xylofuranosyl nucleosides and the lyxo-epoxide" pages 1564-1570, see compound 10 --	21
X	Chemical Abstracts, vol. 72, 1970 (Columbus, Ohio, US) see page 352, abstract no. 3725n & JP, A, 6917910 (DAIICHI SEIYAKU CO. LTD) 06th August 1969 --	21,22
X	Journal of Organic Chemistry, vol. 44, no.8, 1979 (Columbus, Ohio, US) M.J. Robins et al.: "Nucleic acid related compounds.30. Transformations of adenosine to the first 2',3'-aziridine-fused nucleosides, 9-(2,3-epimino-2,3-dideoxy-beta-D-ribofuranosyl)adenine and 9-(2,3-epimino-2,3-dideoxy-beta-D-lyxofuranosyl)adenine" pages 1317-1322, see compound 1 and 7 --	21
A	The Journal of Biological Chemistry, vol. 262, no. 5, 15 February 1987 The American Society of Biological Chemists, Inc. (US) Y.C. Cheng et al.: "Human immuno-deficiency virus reverse transcriptase", pages 2187-2189, see the whole document	22

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers ....*.... because they relate to subject matter not required to be searched by this Authority, namely:

* Claims 1-20
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.